immune response to an antigen, or an ability to withstand stress. Furthermore, the HPA and HPG axes exert such regulatory control via the production of endogenous opioids that interact with opioid in many locations of the body. In particular, in the HPG axis, the mu opioid receptor is centrally involved in tonic regulation of the luteinizing hormone, particularly in its pulsatile release. Furthermore, in the HPA axis, the mu opioid receptor modulates corticotropin releasing factor/hormone (CRF or CRH) in the hypothalamus which in turn modulates production of pro-opiomelanocortin (POMC) in the pituitary which is processed into several active peptides such as ACTH, which stimulates the adrenal cortex to release the stress hormone cortisol in humans, which in turn provides the stress response to environmental stimuli. Furthermore, modulated mu opioid receptor activity can lead to modulation of most cellular and humoral immunity including that mediate through T cells, B cells, cytokines, and chemokines. The pathophysiology of immune disorders may therefore be influenced by pharmacotherapies that modulate the activity of the mu opioid receptor. Moreover, gastrointestinal motility is modulated by modulation of opioid receptor treatment, and diagnosis of a disease or disorder related to gastrointestinal motility (e.g. constipation) may be facilitated by knowledge of intrinsic mu opioid receptor motility.

Applicants have discovered that the binding affinity of an opioid receptor, such as a mu opioid receptor with an endogenous opioid ligand, such as β-endorphin, is expected to modulate such physiological activities. Hence, the binding affinity of variant mu opioid receptors explained above, for endogenous opioid ligands such as β-endorphin, is expected to modulate those physiological activities regulated by the HPA and HPG axes relative to those physiological activities in a standard having mu opioid receptors produced from the predominant or "most common" allele of the mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1. As the result, the present invention extends to a method of diagnosing a disease or disorder related to a physiological function regulated by the HPA or HPG axes. Examples of physiological functions regulated by the HPA and the HPG include, but are not limited to sexual or reproductive functions, gastrointestinal motility, immune response, or ability to withstand stress. Such a method comprises the steps of removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether the first allele comprises a human mu opioid receptor gene comprising a DNA

sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC.

The presence of at least one variation in the human mu opioid receptor gene of the first allele is expected to be indicative of a disorder related to a physiological function regulated by the HPA or GPA, such as sexual or reproductive functions, gastrointestinal motility, immune response, and the ability to withstand stress, wherein the first allele of the standard comprises a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Moreover, a method for diagnosing a disease or disorder related to a physiological function regulated by the HPA or GPA, as described above may further comprise the step of determining whether the second allele of the bodily sample comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC. The presence of the at least one variation in the human mu opioid receptor gene of the second allele of the bodily sample from the subject may be expected to be indicative of a disease or disorder related to sexual and reproductive functions, gastrointestinal motility, immune response, or the ability of the subject to withstand stress.

In another embodiment, the present invention extends to a method for diagnosing a disease or disorder related to a physiological function regulated by the HPA or GPA by examining a bodily sample taken from the subject for the presence of a variant human mu opioid receptor. Such a method comprises the steps of removing a bodily sample comprising a human mu opioid receptor from the subject, and determining whether the human mu opioid receptor present in the sample is a variant human mu opioid receptor of the invention, i.e., comprises an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the variation comprises:

Ser23Pro or conserved variants thereof;

Ser42Thr or conserved variants thereof; or

addition of a Gly residue following Gly63 or conserved variants thereof,

such that the presence of at least one variation is expected to be indicative of a disease or disorder related to a physiological activity regulated by the HPA or HPG axes, such as sexual

function or development, gastric motility, immune response, or the ability of the subject to withstand stress, relative to regulation of such activities in a standard comprising a human mu opioid receptor having an amino acid sequence of SEQ ID NO:2.

Once a disease or disorder related to a physiological function regulated by the HPA or HPG axes has been diagnosed, it is possible for attending medical professionals treating the subject to select and administer an appropriate therapeutic agent and a therapeutically effective amount of the agent to administer to the subject to treat such a disease or disorder. Consequently, the present invention extends to a method for determining an appropriate therapeutic agent to administer to a subject suffering from a disease or disorder related to a physiological function regulated by the HPA or HPG axes, comprising removing a bodily sample from the subject, and determining the presence of at least one variant allele of a mu opioid receptor gene in the bodily sample, wherein the variant allele comprises a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC.

The present invention further extends to a method for selecting an appropriate therapeutic agent to administer to a subject suffering from a disease or disorder related to a physiological function regulated by the HPA or HPG axes as set forth above, further comprising determining whether the bodily sample comprises a second variant allele of the mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC.

The present invention further extends to commercial test kits suitable for use by a medical professional to determine whether either or both alleles of a bodily sample taken from a subject comprise a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC.

Commercial test kits of the present invention have applications in determining susceptibility of pain in the subject relative to a standard. Such kits can also be used to determine a subject's increased or decreased susceptibility to at least one addictive disease relative to susceptibility to

at least one addictive disease in a standard. Also a therapeutically effective amount of pain reliever to administer to the subject in order to induce analgesia in the subject relative to a therapeutically effective amount of pain reliever to administer to a standard to induce analgesia in the standard can be determined. Moreover, a test kit of the present invention has applications in determining a therapeutically effective amount of therapeutic agent for treating at least one addictive disease to administer to a subject suffering from the at least one addictive disease, relative to a therapeutically effective amount of therapeutic agent to administer to a standard suffering from at least one addictive disease. Furthermore, test kits of the invention have applications in diagnosing a disease or disorder related to a physiological condition regulated by the HPA or HPG axes of the neuroendocrine system, and in selecting an appropriate therapeutic agent for treating such a disease or disorder, along with a therapeutically effective amount of agent to administer to the subject. A standard as used herein comprises two alleles of a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Furthermore, a commercial test kit of the present invention can also be used to determine the presence of an isolated variant allele of a human mu opioid receptor gene of the present invention in a bodily sample removed from a subject, which can serve as a genetic marker. As explained above, the predominant or "most common" allele of a human mu opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1. Hence a variant allele comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises:

T67C; T124A; C153T; G174A or 187INSGGC,

or combinations thereof, can be detected in the bodily sample with a commercial kit of the invention.

Other variant alleles of the human mu opioid receptor gene of the present invention can be detected with a commercial test kit of the present invention. For example, an isolated variant allele of a human mu opioid receptor gene detectable with a commercial kit of the present invention, comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein the variations comprise:

T67C; T124A; C153T; G174A or 187INS:GGC.

Accordingly, a commercial test kit may be prepared for determining the presence of at least one variation in a human mu opioid receptor gene of either or both alleles in a bodily sample taken from a subject, wherein the commercial test kit comprises:

a) PCR oligonucleotide primers suitable for detection of an allele comprising a human mu opioid receptor gene having a DNA sequence with a variation in SEQ ID NO:1;

b) other reagents; and

c) directions for use of the kit.

The present invention further extends to commercial test kits capable of detecting a variant human mu opioid receptor in a bodily sample taken from a subject. Examples of variant human mu opioid receptors that can be detected with a kit of the present invention comprise a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises Ser23Pro or conserved variants thereof; Ser42Thr or conserved variants thereof; or a variant human mu opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID NO:2, wherein the variations comprise at least one of :

Ser23Pro or conserved variants thereof;

Ser42Thr or conserved variants thereof; or

addition of a Gly residue or conserved variants thereof.

Moreover, a commercial test kit of the present invention can be used to determine: susceptibility to pain in the subject relative to susceptibility to pain in a standard; a therapeutically effective amount of pain reliever to administer to a subject to induce analgesia in the subject relative to a therapeutically effective amount of pain reliever to administer to a standard to induce analgesia in the standard; a therapeutically effective amount of therapeutic agent for treating at least one addictive disease to administer to a subject suffering from at least one addictive disease, relative to a therapeutically effective amount of therapeutic agent to administer to a standard suffering from the at least one addictive disease;

diagnosing a disease or disorder related to a physiological condition regulated by the HPA or HPG axes of the neuroendocrine system, or selecting an appropriate therapeutic agent for treating such a disease or disorder, along with a therapeutically effective amount of such agent to administer to the subject.

Accordingly, the present invention extends to a commercial test kit having applications set forth above, comprising a predetermined amount of at least one detectably labeled immunochemically reactive component having affinity for a variant human mu opioid receptor;

(b) other reagents; and

(c) directions for use of the kit.

In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive," "sandwich," "double antibody," etc.), and comprises:

(a) a labeled component which has been obtained by coupling the human mu opioid receptor of a bodily sample to a detectable label;

(b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand comprises:

(i) a ligand capable of binding with the labeled component (a);

(ii) a ligand capable of binding with a binding partner of the labeled component (a);

(iii) a ligand capable of binding with at least one of the component(s) to be determined; or

(iv) a ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; or

(c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the human mu opioid receptor gene of the present invention and a specific binding partner thereto.

Accordingly, it is an object of the present invention to provide heretofore unknown variations the DNA sequence of the human mu opioid receptor gene wherein the variations can be used to

map the locus of the human mu opioid receptor gene.

It is yet another object of the present invention to use heretofore unknown polymorphisms of an allele of the human mu opioid receptor gene as markers for any kind of disorder related to the human mu opioid receptor, such as an addictive disease, pain, or markers for genes.

It is another object of the present invention to provide nucleotides, optionally detectably labeled, selectively hybridizable to variant alleles of the human mu opioid receptor gene disclosed herein, as well as polypeptides produced from the expression of the variant alleles and nucleotides selectively hybridizable thereto under selective hybridization conditions.

It is yet another object of the present invention to provide antibodies, optionally detectably labeled, having immunogens comprising polypeptides produced from the expression of variant alleles of human mu opioid receptor gene, or expression of isolated nucleic acid molecules selectively hybridizable to variant alleles disclosed herein.

It is another object of the present invention to gain insight into a subject's susceptibility to pain. This insight can be used to determine a therapeutically effective dose of pain reliever to administer to the subject to induce analgesia therein relative to the therapeutically effective amount of pain reliever administered to a standard to induce analgesia therein, wherein the standard comprises two alleles of the human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1, or a variant human mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2.

Such information can be used to tailor a regimen for treating a subject suffering from at least one addictive disease, relative to the therapeutically effective amount of therapeutic agent administered to a standard suffering from at least one addictive disease.

It is yet another object of the present invention to provide commercial test kits for attending medical professionals to determine the presence of variant alleles of a human mu opioid receptor gene in a bodily sample taken from a subject. The results of such testing can then be

used to determine the subject's susceptibility to pain, susceptibility to at least one addictive disease, determining a therapeutically effective amount of pain reliever to administer to the subject in order to induce analgesia, or determining a therapeutically effective amount of therapeutic agent for treating at least one addictive disease to administer to the subject.

It is an object of the present invention to determine the activity of a mu opioid receptor in a subject, and use such information to diagnose a disease or disorder related to sexual or reproductive function, gastrointestinal motility, immune response, or ability to withstand stress, wherein variant alleles of the mu opioid receptor gene when expressed produce variant mu opioid receptors having activity different from a mu opioid receptor produced from the predominant or "most common" allele of the mu opioid receptor comprising a DNA sequence of SEQ ID NO:1.

It is another object of the present invention to employ Applicants' discovery of a correlation between the activity of a mu opioid and its impact the neuroendocrine system, and particularly on levels of hormones within the body. As a result, the level of activity of the mu opioid receptor effects sexual or reproductive function, gastrointestinal motility, immune response, or ability to withstand stress. Such information can further be used select appropriate therapeutic agents to treat diseases such as infertility, constipation, or diarrhea. Further, such information can be used to select appropriate therapeutic agents to increase immune response against an antigen such as a bacterium, a virus or a tumor cell in the subject, and to treat psychiatric diseases or disorders such as obsessive compulsive disorder, schizophrenia, or depression.

It is yet another object of the present invention to provide commercial detecting variant alleles of the human mu opioid receptor gene or the presence of a variant human mu opioid receptor in a bodily sample taken from a subject. The results of such tests can then be used to gain incite into a subject's ability to withstand pain, susceptibility to addiction, to diagnose a disease or disorder related to a physiological function regulated by the HPA or HPG axes such as sexual and reproductive functions, gastrointestinal motility, immune response, and the ability of the subject to withstand stress.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A - 1B: The nucleic acid (1A) and protein sequence (1B) of the most common allele of the mu opioid receptor (SEQ ID NO:1 and SEQ ID NO:2, respectively) (GENBANK accession number L25119).

Figure 2A - 2B: DNA (2A, SEQ ID NO:3) and protein (2B, SEQ ID NO:4) sequence of the most common allele of the mu opioid receptor with the T67C (Ser23Pro) polymorphism.

Figure 3A - 3B: DNA (3A, SEQ ID NO:5) and protein (3B, SEQ ID NO:6) sequence of the most common allele of the mu opioid receptor with the T124A (Ser42Thr) polymorphism.

Figure 4: DNA sequence (SEQ ID NO:7) of the most common allele of the mu opioid receptor with the C153T polymorphism.

Figure 5: DNA sequence (SEQ ID NO:8) of the most common allele of the mu opioid receptor with the G174A polymorphism.

Figure 6A - 6B: DNA (6A, SEQ ID NO:9) and protein (6B, SEQ ID NO:10) sequence of the most common allele of the mu opioid receptor with the 187INS:GGC polymorphism.

Figure 7A - 7B: Electropherogram of the mu opioid receptor DNA from an individual heterozygous for both the A118G and the T124A single-nucleotide polymorphisms. Figure 6A is the sequence of the (+) strand; figure 7B the (-) strand.

Figure 8A - 8B : Electropherogram of the mu opioid receptor DNA from an individual heterozygous for the C153T single-nucleotide polymorphism. Figure 7A is the sequence of the (+) strand; figure 8B the (-) strand.

Figure 9A - 9B : Electropherogram of the mu opioid receptor DNA from an individual heterozygous for the G174A single-nucleotide polymorphism. Figure 8A is the sequence of the (+) strand; figure 9B the (-) strand.

Figure 10A - 10B : Electropherogram of the mu opioid receptor DNA from an individual heterozygous for the 187INS:GGC polymorphism, in which a GGC codon is inserted after position 187. Figure 10A is the sequence of the (+) strand; figure 10B the (-) strand.

Figure 11A - 11B : Electropherogram of the mu opioid receptor DNA from an individual heterozygous for the T67C (Ser23Pro) polymorphism. Figure 11A is the sequence of the (+) strand; figure 11B the (-) strand.

DETAILED DESCRIPTION OF THE INVENTION

As explained above, the present invention is based upon Applicants' surprising and unexpected discovery of heretofore unknown polymorphisms, including a trinucleotide insertion and single-nucleotide polymorphisms (SNPs), in the human mu opioid receptor, along with combinations thereof. Furthermore, Applicants have discovered that more than one polymorphism can be present in either or both alleles of the human mu opioid receptor gene in a subject.

In addition, the present invention is based upon Applicants' surprising discovery of molecules of heretofore unknown isolated nucleic acid molecules which encode human mu opioid receptors, wherein the DNA sequences include a combination of presently known polymorphisms and subsequently of the human mu opioid receptor polymorphisms discovered by Applicants and set forth herein.

Furthermore, the present invention is based upon Applicants' surprising and unexpected discovery that the expression of variant alleles of the human mu opioid gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variations comprise T67C, T124A or 187INS:GGC, produce a variant mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variations comprise Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, and that these variant receptors

exhibit a binding affinity for β-endorphin that is different from the binding affinity of a mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2, and is encoded by the predominant or "most common" allele of the mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Furthermore, the present invention is based upon Applicants' prediction that variant alleles of the mu opioid receptor gene, which comprise a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A or 187INS:GGC encode variant mu opioid receptors comprising amino acid sequence having a variation in SEQ ID NO:2 wherein the variation comprises Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, the presence of such variant alleles in a bodily sample from a subject is expected to be indicative of the activity of the mu opioid receptors in the subject.

The present invention further extends to heretofore unknown polymorphisms of the human mu opioid receptor gene that can serve as genetic markers to map the locus of the human mu opioid receptor gene.

The present invention extends to diagnostic methods to determine a subject's increased or decreased susceptibility to at least one addictive disease. With the results of such methods, targeted prevention methods, early therapeutic intervention, and improved chronic treatment to opioid addiction are set forth herein and encompassed by the present invention. In addition, attending medical professionals of subjects armed with the results of such diagnostic methods can determine whether administration of opioid analgesics is appropriate or whether non-opioid derived analgesics should be administered to the subject. Also, appropriate choice and type of analgesic can be made in treating a subject's pain.

Methods for determining the presence of the one or more polymorphisms may be made using any of a large variety of methods for identifying altered nucleotides present in a nucleic acid sequence, by way of non-limiting examples as conventional DNA sequencing, differential hybridization to biological chip arrays such as an oligonucleotide gelpad microchip, or single nucleotide extension (SNE) on chip arrays such as on oligonucleotide gelpad microchips.

These methods are known to one of skill in the art, and are merely exemplified by the following citations: Khrapko KR, Lysov YP, Khorlin A, Shick VV, Florentiev VL, Mirzabekov AD. 1989. An oligonucleotide hybridization approach to DNA sequencing. FEBS Lett 256:118-122; Khrapko KR, Lysov YP, Khorlin AA, Ivanov IB, Yershov GM, Vasilenko SL, Florentiev V, Mirzabekov AD, 1991, A method for DNA sequencing by hybridization with oligonucleotide matrix. J DNA sequencing 1: 375-388; Fodor SPA, Read JL, Pirrung MC, Stryer L, Lu AT, Solas, D, 1991, Light directed, spatially addressable parallel chemical synthesis. Science 251:776-773; Southern EM, Maskos U, Elder JK, 1992, Analyzing and comparing nucleic acid sequences by hybridization to arrays of oligonucleotides: evaluation using experimental models, Genomics 13:1008-1017; Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SPA. 1996. Accessing genetic information with high-density DNA arrays. Science 274:610-614; Hacia JG, Brody LC, Chee MS, Fodor SPA, Collins F. 1996. Detection of heterozygous mutations in BCRA1 using high density oligonucleotide arrays and two colour florescence analysis. Nature Genet 14:44-447; Yershov G, Barsky V, Belgovskiy A, Kirillov E, Kreindlin E, Ivanov I, Parinov S, Guschin D, Drobishev A, Dubiley S, Mirzabekov A. 1996. DNA Analysis and diagnostics on oligonucleotide microchips. Proc Natl Acad Sci USA 93:4913-4918; Shick VV Lebed YB, Kryukov GV. 1998. Identification of HLA DQA1 alleles by the oligonucleotide microchip method. Mol Biol 32:697-688. Translated from Molekulyarna Biologiya 32:813-822; Wang DG, Fan J-B, Siao C-J, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipschutz R, Chee M, Lander ES. 1998 Large scale identification, mapping and genotyping of single-nucleotide polymorphisms in the human genome. Science 280:1077-1082; Halushka MK, Fan J-B, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A. 1999. Patterns of single-nucleotide polymorphisms in candidate genes for blood pressure homeostasis. Nature Genet 22:239-247; Cargill M, Altschuler D, Ireland J, Sklar P, Ardlie K, Patil N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. 1999. Characterization of single nucleotide polymorphisms in coding regions of human genes. Nature genet 22;231-238; Parinov S, Barsky V, Yershov G, Kirillov E, Timofeev E, Belgovskiy A, Mirzabekov A. 1996. DNA

sequencing by hybridization to microchip octa- and decanucleotides extended by stacked pentanucleotides. Nucleic Acids Res 24:2998-3004; Guschin D, Yershof G, Zaslavsky A, Gemmell A, Shick V, Proudnikov V, Arenkov P, Mirzabekov A. 1997. Manual manufacturing of oligonucleotide, DNA and protein microchips. Anal Biochem 250:203-211; Drobyshev A, Mologina M. Shik V, Pobedimskaya D, Yershov G, Mirzabekov A. 1997. Sequence analysis by hybridization with oligonucleotide microchip: Identification of b-thalassemia mutations. Gene 188:45-52; Syvänen A-C, Aalto-Setälä K, Harju L, Kontula K, SØderlund H. 1990. A primer-guided nucleotide incorporation assay in the genotyping of apolipoprotein E. Genomics 8:684-692; Pastinen T, Kurg A, Metspalu A, Peltonen L, Syvänen A-C. 1997. Minisequencing: A specific tool for DNA analysis and diagnostics on oligonucleotide arrays. Genome res 7:606-614; Pastinen T, Perola M, Niini P, Terwilliger J, Salomaa V, Vartiainen E, Peltonen L, Syvänen A-C. 1998. Array-based multiplex analysis of candidate gene reveals two independent and additive genetic risk factors for myocardial infarction in the Finnish population. Hum Mol Genet 7:1453-1462; Dubiley S, Kirillov E, Mirzabekov A. 1999. Polymorphism analysis and gene detection by minisequencing on an array of gel-immobilized primers. Nucleic Acids Res 27:e19; and Syvänen A-C. 1999. From gels to chips: "Minisequencing" primer extension analysis of point mutations and single nucleotide polymorphisms. Hum Mutat 13:1-10. Such citations are not intended to be limiting but merely exemplary of the various methods available for detecting one or more of the polymorphisms described herein.

Also, the present invention extends to methods of determining a subject's increased or decreased susceptibility to pain and response to analgesics, and using that information when prescribing analgesics to the subject.

Furthermore, the present invention extends to diagnosing a disease or disorder related to a physiological function regulated by the HPA and HPG axes, such as sexual and reproductive functions, gastrointestinal motility, immune response, and the ability to withstand stress.

The present invention further extends to variant alleles of the human mu opioid receptor gene comprising a DNA sequence comprising a heretofore unknown polymorphism, such as:

T67C; T124A; C153T; G174A or 187INS:GGC, or combinations thereof.

Furthermore, Applicants' invention extends to variant alleles of the human mu opioid receptor gene comprising a DNA sequence having at least two variations in the predominant or "most common" allele comprising a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1, wherein at least one variation comprises T67C; T124A; C153T; G174A or 187INS:GGC, the at least one other being any other of the foregoing or at least one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Furthermore, one aspect of the invention is based upon Applicants' finding that the C187INS:GGC polymorphism has been found only in persons with long-term polydrug abuse and dependency problems.

Consequently, an initial aspect of the present invention involves isolation of heretofore unknown variant alleles of the human mu opioid receptor gene. As used herein, the term "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids.

Furthermore, in accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual,* Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein "Sambrook et al., 1989"); *DNA Cloning: A Practical Approach,* Volumes I and II (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And Translation* [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994).

Therefore, if appearing herein, the following terms shall have the definitions set out below.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, *i.e.*, capable of replication under its own control.

A "cassette" refers to a segment of DNA that can be inserted into a vector at specific restriction sites. The segment of DNA encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation.

A cell has been "transfected" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous DNA when the transfected DNA effects a phenotypic change. Preferably, the transforming DNA should be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

"Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5′ to 3′ direction along the nontranscribed strand of DNA (*i.e.*, the strand

having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (*see* Sambrook et al., *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Polynucleotides capable of discriminating between the wild-type and polymorphic alleles of the invention ("selectively hybridizable") may be prepared, and the conditions under which such polynucleotides selectively hybridize with the polymorphisms of the invention, may be achieved following guidance provided in the art, such as described by Conner et al., 1983, *Proc. Nat. Acad. Sci. U.S.A.* **80**:278-82;Yershov et al., 1996, *Proc. Nat. Acad. Sci. U.S.A.* **93**:4913-18; Drobyshev et al., 1997, *Gene 188*:45-52; and Chee et al., 1996, *Science* **274**:610-614. Selectively hybridizable reporting polynucleotides such as molecular beacons are also well known in the art.

For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.*, 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m, *e.g.*, 40% formamide, with 5x or 6x SSC. High stringency hybridization conditions correspond to the highest T_m, *e.g.*, 50% formamide, 5x or 6x SSC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for selectively hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (*see* Sambrook et al., *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the

position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (*see* Sambrook et al., *supra*, 11.7-11.8). Preferably a minimum length for a selectively hybridizable nucleic acid is at least about 10 nucleotides; preferably at least about 20 nucleotides; and more preferably the length is at least about 30 nucleotides; and most preferably 40 nucleotides. As noted above, the skilled artisan will be guided by the teachings in the art on selecting the length of a polynucleotide or nucleic acid sequence, the position(s) of the variant nucleotide(s), and the conditions and instrumentation to selectively identify nucleic acid sequences comprising one or more of the polymorphisms as described herein.

In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60°C; in a more preferred embodiment, the T_m is 65°C.

"Homologous recombination" refers to the insertion of a foreign DNA sequence of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5′ (amino) terminus and a translation stop codon at the 3′ (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3′ to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

A "promoter sequence" or "promoter" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3′ direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3′ terminus by the transcription initiation site and extends upstream (5′ direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and translated into the protein encoded by the coding sequence.

A coding sequence is "operatively associated with" a transcriptional and translational control sequences, such as a promoter for example, when RNA polymerase transcribes the coding sequence into mRNA, which in turn is translated into a protein encoding by the coding sequence.

A "signal sequence" is included at the beginning of the coding sequence of a protein to be expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to translocate the polypeptide. The term "translocation signal sequence" is used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional in both types of organisms.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the

control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to selectively hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to selectively hybridize therewith and thereby form the template for the synthesis of the extension product.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in

which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

The phrase "expected to be indicative" is used herein to refer to the correlation between the identity of the allelic variation(s) in an individual and the susceptibility of an individual to addictive disease, sensitivity to pain and analgesics, therapeutic effectiveness of analgesics, and other physiological manifestations described herein related to the function of the mu opioid receptor, such as but not limited to the responsiveness to stress, peripheral gastrointestinal function, immune function, and reproductive biology. The correlations are based on the findings in the present invention of the relationship between the biochemistry and cellular function of the variants of the mu opioid receptor and clinical observations, analyzed statistically, on history of drug dependence, reproductive function, gastrointestinal function, response to stress, and other previous or current conditions. Expected correlations of mu opioid receptor alleles and susceptibility to various conditions may be increased susceptibility or decreased susceptibility.

As explained above, within the scope of the present invention are DNA sequences encoding variant alleles of a human mu opioid receptor gene of the present invention, which comprise at least one variation in the predominant or "most common" allele of the human mu opioid receptor gene. The most common allele comprises a DNA sequence of SEQ ID NO:1, and variations in the most common allele comprise:

T67C; T124A; C153T; G174A or 187INS:GGC, or combinations thereof.

In another embodiment, the present invention comprises DNA sequences encoding variant alleles of a human mu opioid receptor gene, comprising at least two variations in the predominant or "most common" allele of the human mu opioid receptor gene, wherein the

most common human mu opioid receptor gene comprises a DNA sequence of SEQ ID NO:1. Variant alleles of the human mu opioid receptor gene encompassed by the present invention comprise a DNA sequence comprising at least two variations of SEQ ID NO:1, wherein one of the variation is T67C; T124; C153T; G174A or 187INS:GGC; and the at least one other is another of the foregoing polymorphisms or one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Moreover, due to degenerate nature of codons in the genetic code, variant human mu opioid receptor proteins encoded by variant alleles of the present invention, wherein the variant human mu opioid receptors comprise an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the variations comprise Ser42Thr or conserved variants thereof; or the addition of a Gly residue following Gly63 or conserved variants thereof, or combinations thereof, or either of the foregoing polymorphisms in combination with the other and/or any known in the art, can be encoded by nucleic acid molecules other than those set forth above. "Degenerate nature" refers to the use of different three-letter codons to specify a particular amino acid pursuant to the genetic code. It is well known in the art that the following codons can be used interchangeably to code for each specific amino acid:

Phenylalanine (Phe or F)	UUU or UUC
Leucine (Leu or L)	UUA or UUG or CUU or CUC or CUA or CUG
Isoleucine (Ile or I)	AUU or AUC or AUA
Methionine (Met or M)	AUG
Valine (Val or V)	GUU or GUC of GUA or GUG
Serine (Ser or S)	UCU or UCC or UCA or UCG or AGU or AGC
Proline (Pro or P)	CCU or CCC or CCA or CCG
Threonine (Thr or T)	ACU or ACC or ACA or ACG
Alanine (Ala or A)	GCU or GCG or GCA or GCG
Tyrosine (Tyr or Y)	UAU or UAC
Histidine (His or H)	CAU or CAC
Glutamine (Gln or Q)	CAA or CAG
Asparagine (Asn or N)	AAU or AAC

Lysine (Lys or K)	AAA or AAG
Aspartic Acid (Asp or D)	GAU or GAC
Glutamic Acid (Glu or E)	GAA or GAG
Cysteine (Cys or C)	UGU or UGC
Arginine (Arg or R)	CGU or CGC or CGA or CGG or AGA or AGG
Glycine (Gly or G)	GGU or GGC or GGA or GGG
Tryptophan (Trp or W)	UGG
Termination codon	UAA (ochre) or UAG (amber) or UGA (opal)

It should be understood that the codons specified above are for RNA sequences. The corresponding codons for DNA have a T substituted for U.

As used herein, the term "sequence homology" in all its grammatical forms refers to the relationship between proteins that possess a "common evolutionary origin," including proteins from superfamilies (*e.g.*, the immunoglobulin superfamily) and homologous proteins from different species (*e.g.*, myosin light chain, etc.) (Reeck et al., 1987, *Cell* **50**:667).

Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that do not share a common evolutionary origin (*see* Reeck et al., *supra*). However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and not a common evolutionary origin.

In a specific embodiment, two DNA sequences are "substantially homologous" or "substantially similar" when at least about 50% (preferably at least about 75%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

Similarly, in a particular embodiment, two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 30% of the amino acids are identical, or greater than about 60% are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7*, Madison, Wisconsin) pileup program.

The term "corresponding to" is used herein to refer to similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is measured. Thus, the term "corresponding to" refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

A variant allele of the human mu opioid receptor gene of the present invention, whether genomic DNA or cDNA, can be isolated from any source, particularly from a human cDNA or genomic library. Methods for obtaining an allele of a human mu opioid receptor gene, variants thereof, or the most common, are well known in the art, as described above (*see, e.g.*, Sambrook et al., 1989, *supra*).

Accordingly, any human cell potentially can serve as the nucleic acid source for the molecular cloning of a variant allele of the human mu opioid receptor gene of the present invention, or a nucleic acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor gene of the present invention. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), and preferably is obtained from a cDNA library prepared from tissues with high level expression of a human mu opioid receptor protein, by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (See, for example, Sambrook et al., 1989, *supra*; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will not contain intron sequences. Whatever the source, an allele of a human mu opioid receptor gene of the present invention should be molecularly cloned into a suitable vector for propagation.

In the molecular cloning of a human mu opioid receptor gene of the present invention, DNA fragments are generated, some of which will encode an allele. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing an allele of a human mu opioid receptor of the present invention may be accomplished in a number of ways. For example, if an amount of a portion of an allele of a human mu opioid receptor gene, or its specific RNA, or a fragment thereof, is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton and Davis, 1977, *Science* **196**:180; Grunstein and Hogness, 1975, *Proc. Natl. Acad. Sci. U.S.A.* **72**:3961). For example, a set of oligonucleotides corresponding to the partial amino acid sequence information obtained for a human mu opioid receptor protein can be prepared and used as probes for DNA encoding a variant allele of a human mu opioid receptor gene of the present invention, as was done in a specific example, *infra*, or as primers for cDNA or mRNA (*e.g.*, in combination with a poly-T primer for RT-PCR). Preferably, a fragment is selected that is highly unique to a variant allele of the human mu opioid receptor gene of the invention. Those DNA fragments with substantial homology to the probe will selectively hybridize. As noted above, the greater the degree of homology, the more stringent hybridization conditions can be used.

Further selection can be carried out on the basis of the properties of an allele of a human mu opioid receptor gene of the present invention *e.g.*, if the allele encodes a variant human mu opioid receptor protein having an isoelectric, electrophoretic, amino acid composition, or partial amino acid sequence different from that produced from the expression of the most common allele of a human mu opioid receptor gene (SEQ ID NO:1) herein. Thus, the presence of an allele of a human mu opioid receptor gene of the present invention may be detected by assays based on the physical, chemical, or immunological properties of its

expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, *e.g.*, has different electrophoretic migration, isoelectric focusing or non-equilibrium pH gel electrophoresis behavior, proteolytic digestion maps, or antigenic properties as known for a human mu opioid receptor produced from expression of a most common allele of the human mu opioid receptor gene (SEQ ID NO:1).

An allele of a human mu opioid receptor gene of the present invention can also be identified by mRNA selection, *i.e.*, by nucleic acid hybridization followed by *in vitro* translation. In this procedure, nucleotide fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified DNA of an allele of a human mu opioid receptor gene of the present invention, or may be synthetic oligonucleotides designed from the partial amino acid sequence information. Immunoprecipitation analysis or functional assays of the *in vitro* translation products of the products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments, that contain the desired sequences.

A labeled cDNA of an allele of a human mu opioid receptor gene of the present invention, or fragments thereof, or a nucleic acid selectively hybridizable to an allele of a human mu opioid receptor gene of the present invention, can be synthesized using sequences set forth herein. The radiolabeled mRNA or cDNA may then be used as a probe to identify homologous DNA fragments from among other genomic DNA fragments. Suitable labels include enzymes, radioactive isotopes, fluorophores (*e.g.*, fluorescein isothiocyanate (FITC), phycoerythrin (PE), Texas red (TR), rhodamine, free or chelated lanthanide series salts, especially Eu^{3+}, to name a few fluorophores), chromophores, radioisotopes, chelating agents, dyes, colloidal gold, latex particles, ligands (*e.g.*, biotin), and chemiluminescent agents. When a control marker is employed, the same or different labels may be used for the receptor and control marker.

In the instance where a radioactive label, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric,

amperometric or gasometric techniques known in the art.

Direct labels are one example of labels which can be used according to the present invention. A direct label has been defined as an entity, which in its natural state, is readily visible, either to the naked eye, or with the aid of an optical filter and/or applied stimulation, e.g., U.V. light to promote fluorescence. Among examples of colored labels, which can be used according to the present invention, include metallic sol particles, for example, gold sol particles such as those described by Leuvering (U.S. Patent 4,313,734); dye sol particles such as described by Gribnau et al. (U.S. Patent 4,373,932) and May et al. (WO 88/08534); dyed latex such as described by May, *supra*, Snyder (EP-a 0 280 559 and 0 281 327); or dyes encapsulated in liposomes as described by Campbell et al. (U.S. Patent 4,703,017). Other direct labels include a radionucleotide, a fluorescent moiety or a luminescent moiety. In addition to these direct labeling devices, indirect labels comprising enzymes can also be used according to the present invention. Various types of enzyme linked immunoassays are well known in the art, for example, alkaline phosphatase and horseradish peroxidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, urease, these and others have been discussed in detail by Eva Engvall in Enzyme Immunoassay ELISA and EMIT in *Methods in Enzymology*, **70**. 419-439, 1980 and in U.S. Patent 4,857,453.

Other labels for use in the invention include magnetic beads or magnetic resonance imaging labels.

Cloning Vectors

The present invention also relates to cloning vectors comprising variant alleles of a human mu opioid receptor gene of the present invention, and an origin of replication. For purposes of this Application, an "origin of replication refers to those DNA sequences that participate in DNA synthesis.

As explained above, in an embodiment of the present invention, variant alleles of a human mu opioid receptor gene of the present invention comprise a DNA sequence having at least one variation in the most common allele of a human mu opioid receptor gene comprising a DNA

sequence of SEQ ID NO:1, wherein the variation comprises T67C; T124A; C153T; G174A or 187INS:GGC, or combinations thereof.

In another embodiment, the present invention extends to variant alleles of a human mu opioid receptor gene, comprising a DNA sequence having at least two variations in the DNA sequence of SEQ ID NO:1, wherein one of the variations comprises T67C; T124A; C153T; G174A or 187INS:GGC, the at least one other being another of the foregoing or one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Furthermore, an isolated variant allele of a human mu opioid receptor gene of the present invention, or isolated nucleic acid molecules selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene of the present invention, can be inserted into an appropriate cloning vector in order to produce multiple copies of the variant allele or isolated nucleic acid molecule. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses. The vector system used however must be compatible with the host cell used. Examples of vectors include having applications herein, but are not limited to *E. coli*, bacteriophages such as lambda derivatives, or plasmids such as pBR322 derivatives or pUC plasmid derivatives, *e.g.*, pGEX vectors, pmal-c, pFLAG, etc. The insertion into a cloning vector can, for example, be accomplished by ligating a variant allele of the human mu opioid receptor gene of the present invention, or an isolated nucleic acid selectively hybridizable thereto, into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the variant allele or isolated nucleic acid selectively hybridizable thereto are not present in the cloning vector, the ends of the variant allele or the isolated nucleic acid molecule selectively hybridizable thereto may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. Such recombinant molecules can then be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of a variant allele of a human mu opioid receptor gene of the present invention, or an isolated nucleic acid molecule selectively hybridizable thereto, can be

generated. Preferably, the cloned isolated variant is contained on a shuttle vector plasmid, which provides for expansion in a cloning cell, *e.g.*, *E. coli*, and facile purification for subsequent insertion into an appropriate expression cell line, if such is desired. For example, a shuttle vector, which is a vector that can replicate in more than one type of organism, can be prepared for replication in both *E. coli* and *Saccharomyces cerevisiae* by linking sequences from an *E. coli* plasmid with sequences from the yeast 2μ plasmid.

In an alternative method an isolated variant allele of a human mu opioid receptor gene of the present invention or an isolated nucleic acid molecule selectively hybridizable thereto may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for a variant allele, for example, by size fractionation, can be done before insertion into the cloning vector.

Expression Vectors

As stated above, the present invention extends to an isolated variant allele of a human mu opioid receptor gene, comprising a DNA sequence having at least one variation in the DNA sequence of the predominant or "most common" allele of the human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1 wherein the variations comprise T67C; T124A; C153T; G174A or 187INSGGC, or combinations thereof.

In another embodiment, the present invention extends to an isolated variant allele of a human mu opioid receptor gene, a DNA sequence having at least two variations in the predominant or "most common" allele of the human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1 wherein the at least one variation is T67C; T124A; C153T; G174A or 187INSGGC, the at least one other being another of the foregoing or a variant known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.

Variant alleles of the present invention, along with isolated nucleic acid molecules selectively hybridizable to such variant alleles, can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Thus, a variant allele of the present invention, or an isolated

nucleic acid molecule selectively hybridizable to a variant allele of the present invention, is operatively associated with a promoter in an expression vector of the invention. A DNA sequence is "operatively associated" to an expression control sequence, such as a promoter, when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively associated" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a variant allele of the present invention, or an isolated nucleic acid selectively hybridizable thereto does not contain an appropriate start signal, such a start signal can be inserted into the expression vector in front of (5' of) the molecule.

Both cDNA and genomic sequences can be cloned and expressed under control of such regulatory sequences. An expression vector also preferably includes a replication origin.

The necessary transcriptional and translational signals can be provided on a recombinant expression vector, or they may be supplied by an allele comprising a human mu opioid receptor gene.

Potential host-vector systems include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.
A variant allele of a human mu opioid receptor gene of the present invention or an isolated nucleic acid molecule selectively hybridizable thereto may be expressed chromosomally, after integration of the coding sequence by recombination. In this regard, any of a number of amplification systems may be used to achieve high levels of stable gene expression (*See* Sambrook et al., 1989, *supra*).

A unicellular host transformed or transfected with an expression vector of the present invention is cultured in an appropriate cell culture medium that provides for expression by the unicellular host of the variant allele, or isolated nucleic acid selectively hybridizable thereto.

Any of the methods previously described for the insertion of DNA fragments into a cloning vector may be used to construct expression vectors of the present invention. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombination (genetic recombination).

Expression of a variant allele of a human mu opioid receptor gene of the present invention or an isolated nucleic acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor gene, may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. Promoters which may be used to control expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, *Nature* **290**:304-310), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol.

7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadal releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Moreover, expression vectors comprising a variant allele of a human mu opioid receptor gene of the present invention, or an isolated nucleic acid molecule selectively hybridizable thereto, can be identified by four general approaches: (a) PCR amplification of the desired plasmid DNA or specific mRNA, (b) nucleic acid hybridization, (c) presence or absence of selection marker gene functions, and (d) expression of inserted sequences. In the first approach, the variant allele or isolated nucleic acid molecule selectively hybridizable thereto can be amplified by PCR to provide for detection of the amplified product. In the second approach, the presence of a foreign gene inserted into an expression vector of the present invention can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted marker gene. In the third approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "selection marker" gene functions (*e.g.*, β-galactosidase activity, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. In yet another example, if an isolated variant allele of a human mu opioid receptor gene of the present invention, or an isolated nucleic acid molecule selectively hybridizable thereto, is inserted within the "selection marker" gene sequence of the vector, recombinants containing the insert can be identified by the absence of the inserted gene function. In the fourth approach, recombinant expression vectors can be identified by assaying

for the activity, biochemical, or immunological characteristics of the gene product expressed by the recombinant, provided that the expressed protein assumes a functionally active conformation.

Naturally, the present invention extends to a method of producing a variant human mu opioid receptor comprising an amino acid sequence having at least one variation in the amino acid sequence of SEQ ID NO:2, wherein the variation comprises Ser23Pro or conserved variants thereof. An example of such a method comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, wherein the variant allele which is operatively associated with a promoter. The transformed or transfected unicellular host is then cultured under conditions that provide for expression of the variant allele of the human mu opioid receptor gene, and the expression product is recovered from the unicellular host.

Another example involves culturing a unicellular host transformed or transfected with an isolated nucleic acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises T67C, wherein the isolated nucleic acid molecule is operatively associated with a promoter. The variant human mu opioid receptor is then recovered from the host.

Furthermore, the present invention extends to a method of producing a variant human mu opioid receptor comprising an amino acid sequence having at least one variation in the amino acid sequence of SEQ ID NO:2, wherein the variation comprises Ser42Thr or conserved variants thereof. An example of such a method comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T124A, wherein the variant allele which is operatively associated with a promoter. The transformed or transfected unicellular host is then cultured under conditions that provide for expression of the variant allele of the human mu opioid

receptor gene, and the expression product is recovered from the unicellular host.

Another example involves culturing a unicellular host transformed or transfected with an isolated nucleic acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises T124A, wherein the isolated nucleic acid molecule is operatively associated with a promoter. The variant human mu opioid receptor is then recovered from the host.

And further, the present invention extends to a method of producing a variant human mu opioid receptor comprising an amino acid sequence having at least one variation in the amino acid sequence of SEQ ID NO:2, wherein the variation comprises 187INS:GGC or conserved variants thereof. An example of such a method comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises the addition of a glycine residue following Gly63, wherein the variant allele which is operatively associated with a promoter. The transformed or transfected unicellular host is then cultured under conditions that provide for expression of the variant allele of the human mu opioid receptor gene, and the expression product is recovered from the unicellular host.

Another example involves culturing a unicellular host transformed or transfected with an isolated nucleic acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises 187INS:GGC, wherein the isolated nucleic acid molecule is operatively associated with a promoter. The variant human mu opioid receptor is then recovered from the host.

In another embodiment, the present invention extends to a method for producing a variant human mu opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID NO:2, wherein the variations comprise

Ser23Pro or conserved variants thereof;

Ser42Thr or conserved variants thereof;

addition of a Gly residue following Gly63 or conserved variants thereof;

Such a method comprises the steps of culturing a unicellular host transformed or transfected with an expression vector comprising a variant allele of a human mu opioid receptor gene of the present invention or an isolated nucleic acid molecule selectively hybridizable thereto, and operatively associated with a promoter, that provides for expression of the variant allele or the isolated nucleic acid molecule selectively hybridizable thereto . After expression, a variant human mu opioid receptor of the present invention is recovered from the unicellular host.

A wide variety of unicellular host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *E. coli* plasmids col El, pCR1, pBR322, pMal-C2, pET, pGEX (Smith *et al.*, 1988, Gene 67:31-40), pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage λ, e.g., NM989, and other phage DNA, e.g., M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

For example, in a baculovirus expression systems, both non-fusion transfer vectors, such as but not limited to pVL941 (*Bam*H1 cloning site; Summers), pVL1393 (*Bam*H1, *Sma*I, *Xba*I, *Eco*R1, *Not*I, *Xma*III, *Bgl*II, and *Pst*I cloning site; Invitrogen), pVL1392 (*Bgl*II, *Pst*I, *Not*I, *Xma*III, *Eco*RI, *Xba*I, *Sma*I, and *Bam*H1 cloning site; Summers and Invitrogen), and pBlue*Bac*III (*Bam*H1, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, with blue/white recombinant screening possible; Invitrogen), and fusion transfer vectors, such as but not limited to pAc700 (*Bam*H1 and *Kpn*I cloning site, in which the *Bam*H1 recognition site begins with the initiation codon; Summers), pAc701 and pAc702 (same as pAc700, with different reading frames),

pAc360 (*Bam*H1 cloning site 36 base pairs downstream of a polyhedrin initiation codon; Invitrogen(195)), and pBlueBacHisA, B, C (three different reading frames, with *Bam*H1, *Bgl*II, *Pst*I, *Nco*I, and *Hind*III cloning site, an N-terminal peptide for ProBond purification, and blue/white recombinant screening of plaques; Invitrogen (220)) can be used.

Mammalian expression vectors contemplated for use in the invention include vectors with inducible promoters, such as the dihydrofolate reductase (DHFR) promoter, *e.g.*, any expression vector with a *DHFR* expression vector, or a *DHFR*/methotrexate co-amplification vector, such as pED *Pst*I, *Sal*I, *Sba*I, *Sma*I, and *Eco*RI cloning site, with the vector expressing both the cloned gene and *DHFR*; *see* Kaufman, *Current Protocols in Molecular Biology*, 16.12 (1991).

Alternatively, a glutamine synthetase/methionine sulfoximine co-amplification vector, such as pEE14 (*Hind*III, *Xba*I, *Sma*I, *Sba*I, *Eco*RI, and *Bcl*I cloning site, in which the vector expresses glutamine synthase and the cloned gene; Celltech). In another embodiment, a vector that directs episomal expression under control of Epstein Barr Virus (EBV) can be used, such as pREP4 (*Bam*H1, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*II, and *Kpn*I cloning site, constitutive RSV-LTR promoter, hygromycin selectable marker; Invitrogen), pCEP4 (*Bam*H1, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*II, and *Kpn*I cloning site, constitutive hCMV immediate early gene, hygromycin selectable marker; Invitrogen), pMEP4 (*Kpn*I, *Pvu*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I, *Sfi*I, *Bam*H1 cloning site, inducible metallothionein IIa gene promoter, hygromycin selectable marker: Invitrogen), pREP8 (*Bam*H1, *Xho*I, *Not*I, *Hind*III, *Nhe*I, and *Kpn*I cloning site, RSV-LTR promoter, histidinol selectable marker; Invitrogen), pREP9 (*Kpn*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I, *Sfi*I, and BamHI cloning site, RSV-LTR promoter, G418 selectable marker; Invitrogen), and pEBVHis (RSV-LTR promoter, hygromycin selectable marker, N-terminal peptide purifiable via ProBond resin and cleaved by enterokinase; Invitrogen). Selectable mammalian expression vectors for use in the invention include pRc/CMV (*Hind*III, *Bst*XI, *Not*I, *Sba*I, and *Apa*I cloning site, G418 selection; Invitrogen), pRc/RSV (*Hind*III, *Spe*I, *Bst*XI, *Not*I, *Xba*I cloning site, G418 selection; Invitrogen), and others. Vaccinia virus mammalian expression vectors (*see*, Kaufman, 1991, *supra*) for use according to the invention include but are not limited to pSC11 (*Sma*I cloning site, TK- and β-

gal selection), pMJ601 (*Sal*I, *Sma*I, *Afl*I, *Nar*I, *Bsp*MII, *Bam*HI, *Apa*I, *Nhe*I, *Sac*II, *Kpn*I, and *Hind*III cloning site; TK- and β-gal selection), and pTKgptF1S (*Eco*RI, *Pst*I, *Sal*I, *Acc*I, *Hind*II, *Sba*I, *Bam*HI, and Hpa cloning site, TK or XPRT selection).

Yeast expression systems can also be used according to the invention to produce a variant human mu opioid receptor or the present invention. For example, the non-fusion pYES2 vector (*Xba*I, *Sph*I, *Sho*I, *Not*I, *Gst*XI, *Eco*RI, *Bst*XI, *Bam*H1, *Sac*I, *Kpn*1, and *Hind*III cloning sit; Invitrogen) or the fusion pYESHisA, B, C (*Xba*I, *Sph*I, *Sho*I, *Not*I, *Bst*XI, *Eco*RI, *Bam*H1, *Sac*I, *Kpn*I, and *Hind*III cloning site, N-terminal peptide purified with ProBond resin and cleaved with enterokinase; Invitrogen), to mention just two, can be employed according to the invention.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

Examples of unicellular hosts contemplated by the present invention include, but are not limited to *E. coli* Pseudonomas, Bacillus, Streptomyces, yeast, CHO, R1.1, B-W, L-M, COS1, COS7, BSC1, BSC40, BMT10 and Sf9 cells. In addition, a host cell strain may be chosen which modulates the expression of a variant allele comprising a human mu opioid receptor gene, or an isolated nucleic acid selectively hybridizable thereto, such that the gene product is modified and processed in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage [*e.g.*, of signal sequence]) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an nonglycosylated core protein product. However, a translocation signal sequence of an isolated

variant allele of a human mu opioid receptor gene of the present invention, or an isolated nucleic acid selectively hybridizable thereto, expressed in bacteria may not be properly spliced. Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding. Moreover, expression in mammalian cells can provide a tool for reconstituting, or constituting activity of the variant human mu opioid receptor gene. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent.

Vectors are introduced into the desired unicellular hosts by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

An isolated variant human mu opioid receptor of the present invention produced as an integral membrane protein can be isolated and purified by standard methods. Generally, the variant human mu opioid receptor can be obtained by lysing the membrane with detergents, such as but not limited to, sodium dodecyl sulfate (SDS), Triton X-100, Nonidet P-40 (NP-40), digoxin, sodium deoxycholate, and the like, including mixtures thereof. Solubilization can be enhanced by sonication of the suspension. Soluble forms of an isolated variant of a human mu opioid receptor can be obtained by collecting culture fluid, or solubilizing inclusion bodies, *e.g.*, by treatment with detergent, and if desired sonication or other mechanical processes, as described above. The solubilized or soluble protein can be isolated using various techniques, such as polyacrylamide gel electrophoresis (PAGE), isoelectric focusing, 2-dimensional gel electrophoresis, chromatography (*e.g.*, ion exchange, affinity, immunoaffinity, and sizing column chromatography), centrifugation, differential solubility, immunoprecipitation, or by any other standard technique for the purification of proteins.

Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode the variant human mu opioid receptors of the present invention may be used in the practice of the

present invention. These include but are not limited to allelic genes, homologous genes from other species, and nucleotide sequences comprising all or portions of genes which are altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Likewise, the conserved variants of human mu opioid receptors of the present invention include, but are not limited to, those containing, as a primary amino acid sequence, substitutions of amino acids in a variant human mu opioid receptor as set forth above, which are functionally equivalent to amino acids of the variations set forth above, resulting in a conservative amino acid substitution. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH_2 can be maintained.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced at a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β-turns in the

protein's structure.

Antibodies to Variant Human mu Opioid Receptors of the Present Invention

According to the invention, variant human mu opioid receptors disclosed herein may be used as an immunogen to generate antibodies that recognize the claimed variant mu opioid receptors. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. Furthermore, antibodies of the invention may be cross reactive, *e.g.*, they may recognize human mu opioid receptors comprising an amino acid sequence of SEQ ID NO:1, as well as mu opioid receptors from different species. Polyclonal antibodies have greater likelihood of cross reactivity. Alternatively, an antibody of the invention may be specific for a specific variant allele of a mu opioid receptor.

Various procedures known in the art may be used for the production of polyclonal antibodies to variant opioid receptors disclosed herein. For the production of an antibody, various host animals can be immunized by injection with a variant human mu opioid receptor of the invention, including but not limited to rabbits, mice, rats, sheep, goats, etc. In one embodiment, the variant human mu opioid receptor can be conjugated to an immunogenic carrier, *e.g.*, bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed toward a particular human mu opioid receptor of the present invention, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler and Milstein [*Nature* **256**:495-497 (1975)], as well as the trioma technique, the human B-cell hybridoma technique [Kozbor et al., *Immunology Today* **4**:72 1983); Cote et al., *Proc. Natl. Acad. Sci. U.S.A.* **80**:2026-2030

(1983)], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)]. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology [PCT/US90/02545]. In fact, according to the invention, techniques developed for the production of "chimeric antibodies" [Morrison et al., *J. Bacteriol.* **159**:870 (1984); Neuberger et al., *Nature* **312**:604-608 (1984); Takeda et al., *Nature* **314**:452-454 (1985)] by splicing the genes from a mouse antibody molecule specific for a variant human mu opioid receptor of the present invention together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention. Such human or humanized chimeric antibodies are preferred for use in determining the presence of a particular human mu opioid receptor in a sample taken from a subject.

According to the invention, techniques described for the production of single chain antibodies [U.S. Patent Nos. 5,476,786 and 5,132,405 to Huston; U.S. Patent 4,946,778] can be adapted to produce particular variant mu opioid receptor-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries [Huse et al., *Science* **246**:1275-1281 (1989)] to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for a variant mu opioid receptor of the present invention.

Antibody fragments which contain the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin

reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. For example, to select antibodies which recognize a specific epitope of a variant human mu opioid receptor of the present invention, one may assay generated hybridomas for a product which binds to a fragment of the variant human mu opioid receptor containing such epitope.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of a variant human mu opioid receptor, *e.g.*, for Western blotting, imaging a variant human mu opioid receptor *in situ*, measuring levels thereof in appropriate physiological samples, etc. using any of the detection techniques mentioned above or known in the art.

Consequently, the present invention extends to a method for determining a susceptibility of a subject to one addictive disease comprising removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether either the first or second alleles, or both alleles comprise a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises:

T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination thereof.

In this embodiment, the biological sample can be a biological fluid, such as but not limited to, blood, serum, plasma, interstitial fluid, plural effusions, urine, cerebrospinal fluid, and the like. Preferably, variant alleles of a human mu opioid receptor gene, as described above, are detected in serum or urine, which are both readily obtained. Alternatively, variant alleles of a human mu opioid receptor gene indicating increased or decrease susceptibility to addictive diseases in the subject as described above, can be detected from cellular sources, such as, but

not limited to, brain tissue biopsies, adipocytes, testes, heart, and the like. For example, cells can be obtained from an individual by biopsy and lysed, *e.g.*, by freeze-thaw cycling, or treatment with a mild cytolytic detergent such as, but not limited to, TRITON X-100®, digitonin, NONIDET P (NP)-40®, saponin, and the like, or combinations thereof (*see, e.g.*, International Patent Publication WO 92/08981, published May 29, 1992). In yet another embodiment, samples containing both cells and body fluids can be used (*see ibid.*).

Other methods presently understood by a skilled artisan, and encompassed by the present invention, can also be used to detect the presence of either variation in either or both alleles of a human mu opioid receptor gene in a sample, and hence increased or decreased susceptibility to at least one addictive disease of the subject relative to the susceptibility of at least one addictive disease in a standard comprising alleles of the human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

For example, an optionally detectably labeled isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T124A, can be used in standard Northern hybridization analysis to detect the presence, and in some instances quantitate the level of transcription of such a variant allele of the present invention.

Alternatively, oligonucleotides of the invention can be used as PCR primers to amplify an allele of a human mu opioid receptor gene of the biological sample *e.g.*, by reverse transcriptase-PCR, or amplification of the allele itself. The amplified mRNA or DNA can then be quantified or sequenced in order to determine the presence of a variant allele, and the susceptibility of the subject to addictive diseases. Furthermore, variations in SEQ ID NO:1, as described above, can be found by creation or deletion of restriction fragment length polymorphisms (RFLPs) not found in the predominant or "most common" allele, hybridization with a specific probe engineered to selectively hybridize to variation described, (or lack of hybridization with a probe specific for the predominant or "most common" allele), as well as by other techniques.

Furthermore, biochemical or immunochemical/biochemical (*e.g.*, immunoprecipitation) techniques can be used to detect the presence and or level of expression of a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A or 187INS:GGC.

For example, methods such as radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc using antibodies of the present invention, can be used to determine the presence of a variant in an allele of a human mu opioid receptor gene in a sample taken from the subject, and hence, the subject's susceptibility to addictive diseases relative to the susceptibility of a standard. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

Determining susceptibility to pain in a Subject

In yet another embodiment, the present invention extends to a method for determining a susceptibility to pain in a subject.

Hence, disclosed herein is a method of determining susceptibility of pain in a subject, comprising the steps of removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether either the first or second alleles, or both alleles, comprise a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A or187INS:GGC.

The presence of at least one variation in either or both alleles of the human mu opioid receptor

gene is expected to be indicative of the subject's increased or decreased susceptibility to pain relative to a person homozygous with respect to the predominant or "most common" allele comprising a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Numerous methods presently available, and understood by the skilled artisan, can be used to "genotype" a subject in regards to the presence of a variant allele of a human mu opioid receptor gene in the genome of the subject. In particular, methods described above to ascertain increased or decreased susceptibility to addictive diseases have relevance in this embodiment of the present invention, and can readily be used herein. For example, Northern blot hybridization an isolated nucleic acid of the present invention selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation of SEQ ID NO:1, wherein the variation comprises T67C; T124A; or187INS:GGC, as a probe, along with RT-PCR, PCR, and numerous immunoassays described above, have applications herein.

Moreover, once susceptibility to pain in a subject has been determined, it is possible for attending medical professionals treating the subject for pain to administer an appropriate amount of pain reliever to the subject in order to induce analgesia. More specifically, an inappropriate amount of pain reliever is administered to a subject when either the subject is not relieved of pain, or the subject is exposed to potential deleterious side effects of the pain reliever, such as induction of addiction to the pain reliever, brain damage, or death.

However, since the amount of pain reliever administered to a subject is presently based principally on weight, information regarding the genotype of the subject with respect to the human mu opioid receptor gene can help increase accuracy in determining a therapeutically effective amount of pain reliever to administer in order to induce analgesia, making the use of pain relievers much safer for the subject.

Similarly, once ascertained, a susceptibility to addiction and response to human mu opioid receptor directed therapeutic agents, appropriate medications and dosages thereof can be determined for treatment of addictive diseases.

Diagnosing and treating a disease or disorder related to a physiological function regulated by the HPA or HPG axes

In yet another embodiment, the present invention extends to a method for diagnosing a disease or disorder related to a physiological function regulated by the HPA or HPG axes. Examples of such physiological functions include sexual or reproductive functions, gastrointestinal motility, immune response, or ability to withstand stress. Moreover, examples of diseases or disorders which can be diagnosed with the present invention include infertility, constipation, diarrhea, and decreased immune response to name only a few.

Hence, disclosed herein is a method of diagnosing a disease or disorder related to a physiological function regulated by the HPA or HPG axes in a subject, comprising the steps of removing a bodily sample comprising a first and second allele of a human mu opioid receptor gene from the subject, and determining whether either the first or second alleles, or both alleles, comprise a DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises T67C; T124A or187INS:GGC.

The presence of at least one variation in either or both alleles of the human mu opioid receptor gene is expected to be indicative of a disease or disorder related to a physiological function regulated by the HPA or HPG axes relative to such functions in a person homozygous with respect to the predominant or "most common" allele comprising a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1. Examples of such physiological functions include sexual or reproductive functions, gastrointestinal motility, immune response, or ability to withstand stress. Moreover, examples of diseases or disorders which can be diagnosed with the present invention include infertility, constipation, diarrhea, and decreased immune response to name only a few. relative to a person homozygous with respect to the predominant or "most common" allele comprising a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Numerous methods presently available, and understood by the skilled artisan, can be used to "genotype" a subject in regards to the presence of a variant allele of a human mu opioid receptor gene in the genome of the subject. In particular, methods described above to ascertain

increased or decreased susceptibility to addictive diseases have relevance in this embodiment of the present invention, and can readily be used herein. For example, Northern blot hybridization an isolated nucleic acid of the present invention selectively hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation of SEQ ID NO:1, wherein the variation comprises T67C; T124A; or187INS:GGC as a probe, along with RT-PCR, PCR, and numerous immunoassays described above, have applications herein.

In an alternative, such a method comprises removing a bodily sample from the subject comprising a mu opioid receptor, and determining whether the receptor comprises an amino acid sequence having a variation in SEQ ID NO:1, wherein the variation comprises: Ser23Pro, Ser42Thr or conserved variants thereof; or addition of a Gly residue following Gly 63 or conserved variants thereof, such that the presence of at least one variation is expected to be indicative of a disease or disorder related to a physiological function regulated by the HPA or HPG axes, such as sexual function or development, gastric motility, immune response, or the ability of the subject to withstand stress, relative to regulation of such activities in a standard comprises a human mu opioid receptor having an amino acid sequence of SEQ ID NO:2.

In particular, the presence of a variant human mu opioid receptor comprising an amino acid sequence having at least one variation in SEQ ID NO:2 wherein the variation comprises Ser23Pro or conserved variants thereof, is expected to be indicative of increased sexual or reproductive functions, increased gastrointestinal motility, increased immune response, or increased ability to withstand stress relative to the levels of such function observed in a standard having a mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2.

Moreover, the presence of a variant human mu opioid receptor comprising an amino acid sequence having at least one variation in SEQ ID NO:2 wherein the variation comprises Ser42Thr or conserved variants thereof, is expected to be indicative of increased sexual or reproductive functions, increased gastrointestinal motility, increased immune response, or increased ability to withstand stress relative to the levels of such function observed in a

standard having a mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2.

Furthermore, the presence of a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises the addition of a Gly residue following Gly63 or conserved variants thereof, in a bodily sample taken from a subject is expected to be indicative of decreased sexual or reproductive functions, decreased gastrointestinal motility, decreased immune response, or decreased ability to withstand stress relative to the levels of such function observed in a standard having a mu opioid receptor comprising an amino acid sequence of SEQ ID NO:2. Examples of specific diseases or disorders related to regulation of physiological functions regulated by the HPA or HPG axes include infertility, constipation, diarrhea, decreased immune response to antigens, or a lack of ability to withstand stress.

Numerous methods of detecting a variant mu opioid receptor as described above are presently available to the skilled artisan. For example a receptor in the bodily sample can be digested into fragments with proteases or CNBr. These fragments can then be collected and sequenced using presently known methods. Once the sequence of the receptor has been determined, it is a simple matter of comparing it to the amino acid sequence of the predominant or "most common" receptor having an amino acid sequence of SEQ ID NO:2, to determine whether a variation in the amino acid sequence exists. Other methods involve immune assays described herein using antibodies of the present invention, or a binding assay to determine the binding affinity of the receptor to β-endorphin.

Moreover, once a disease or disorder related to a physiological condition regulated by the HPA or HPG axes has been diagnosed, it is possible for attending medical professionals treating the suspect to select an appropriate therapeutic agent for treating such a disease and disorder, and a therapeutically effective amount of such pain reliever to administer to the subject. Hence naturally, the present invention extends to a method for selecting an appropriate therapeutic agent for treating a disease or disorder related to a physiological function regulated by the HPA and HPG axes, wherein such physiological functions include sexual and reproductive functions, gastrointestinal motility, immune response, and ability to withstand stress. Furthermore,

diseases or disorders related to such functions which can be diagnosed with the present invention include, but are not limited to, infertility, constipation, diarrhea, and decreased immune response, to name only a few.

Commercial Kits

Furthermore, as explained above, the present invention extends to commercial kits having applications in screening a bodily sample taken from a subject for the presence of a variant allele comprising a human mu opioid receptor comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A or187INS:GGC, or combinations thereof, as well as with other known polymorphisms.

With information obtained from the use of a test kit of the present invention, an attending health profession can determine whether the subject has an susceptibility to pain relative to a standard, an increased susceptibility to at least one addictive disease relative to the susceptibility of a standard, a therapeutically effective amount of pain reliever to administer to the subject suffering from pain in order to induce analgesia in the subject relative to the therapeutically effective amount of pain reliever to administer to a standard in order to induce analgesia in the standard, or a therapeutically effective amount therapeutic agent to administer to a subject suffering from at least one addictive disease, relative to the therapeutically effective amount of therapeutic agent to administer to standard suffering from at least one addictive disease. Furthermore, such information can also be used to diagnose a disease or disorder related to a physiological function regulated by the HPA or HPG axes, such as sexual or reproductive functions, gastrointestinal motility, immune response, or ability to withstand stress, or selecting an appropriate therapeutic agent and a therapeutically effective amount of such an agent to administer to a subject suffering from a disease or disorder related to a physiological function regulated by the HPA or HPG axes. In each use described above, the standard comprises a first and or second allele of a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Accordingly, a test kit of the present invention for determining whether a subject comprises a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a

variation in SEQ ID NO:1, comprises means for detecting the presence of a variation in a first and or second allele comprising a human mu opioid receptor in a biological sample from a subject, and optimally packaged with directions for use of the kit. In one particular aspect, the means for detecting the presence of a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, comprises a specific binding partner of a human mu opioid receptor, such as an antibody, and means for detecting the level of binding of the specific binding partner of the antibody to the particular human mu opioid receptor. In another embodiment, a test kit comprises an oligonucleotide probe(s) for binding to a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1; and means for detecting the level of binding of the probe to the variant allele, wherein detection binding of the probe to the variant allele indicates the presence of a variant comprising a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, wherein the variation comprises: T67C, T124A, C153T, G174A or187INS:GGC, or combinations thereof, as well as in combination with other known polymorphisms.

The sequence of the oligonucleotide probe used in a commercial kit will determine which if any variation is present in an allele comprising a human mu opioid receptor gene. Should no binding be detected, it is probable that no such variation exists in either allele of the subject.

More specifically, a commercial test kit of the present invention comprises:

a) PCR oligonucleotide primers suitable for detection of a variant allele of a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1, as set forth above,

b) other reagents; and

c) directions for use of the kit.

Examples of PCR oligonucleotide primer suitable for detection of an allele comprising a human mu opioid receptor gene comprising a DNA sequence having a variation in SEQ ID NO:1 can be readily produced by a person of ordinary skill in the art with teaching set forth herein, and variations of SEQ ID NO:1 also set forth herein.

The present invention further extends to commercial test kits capable of detecting a variant human mu opioid receptor in a bodily sample taken from a subject. Examples of variant human mu opioid receptors that can be detected with a kit of the present invention comprise:

(a) a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises the variation comprises Ser23Pro or conserved variants thereof;

(b) a variant human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises the variation comprises Ser42Thr or conserved variants thereof; or

(c) a variant human mu opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID NO:2, wherein the variations comprise the addition of a Gly residue following Gly63 or conserved variants thereof.

Moreover, a commercial test kit of the present invention can be used to determine: a susceptibility to pain in a subject relative to a standard, an increased susceptibility to at least one addictive disease in a subject relative to the susceptibility of a standard, a therapeutically effective amount of pain reliever to administer to the subject suffering from pain in order to induce analgesia in the subject relative to the therapeutically effective amount of pain reliever to administer to a standard in order to induce analgesia in the standard, a therapeutically effective amount of a therapeutic agent to administer to a subject suffering from at least one addictive disease, relative to the therapeutically effective amount of therapeutic agent to administer to standard suffering from at least one addictive disease, a diagnosis of a disease or disorder related to a physiological function regulated by the HPA or HPG axes, such as sexual or reproductive functions, gastrointestinal motility, immune response, or ability to withstand stress, or selecting an appropriate therapeutic agent and a therapeutically effective amount of such an agent to administer to a subject suffering from a disease or disorder related to a physiological function regulated by the HPA or HPG axes. In each use described above, the standard comprises a first and or second allele of a human mu opioid receptor gene comprising a DNA sequence of SEQ ID NO:1.

Accordingly, the present invention extends to a commercial test kit having applications set forth

above, comprising a predetermined amount of at least one detectably labeled immunochemically reactive component having affinity for a variant human mu opioid receptor;

(b) other reagents; and

(c) directions for use of the kit.

Antibodies of the present invention, and set forth above, have readily applications in a commercial test kit of the present invention.

In a further variation, the test kit may be prepared and used for the purposes stated above, which operates according to a predetermined protocol (e.g. "competitive," "sandwich," "double antibody," etc.), and comprises:

(a) a labeled component which has been obtained by coupling the human mu opioid receptor of a bodily sample to a detectable label;

(b) one or more additional immunochemical reagents of which at least one reagent is a ligand or an immobilized ligand, which ligand is selected from the group consisting of:

(i) a ligand capable of binding with the labeled component (a);

(ii) a ligand capable of binding with a binding partner of the labeled component (a);

(iii) ligand capable of binding with at least one of the component(s) to be determined; and

(iv) ligand capable of binding with at least one of the binding partners of at least one of the component(s) to be determined; and

(c) directions for the performance of a protocol for the detection and/or determination of one or more components of an immunochemical reaction between the human mu opioid receptor gene of the present invention and a specific binding partner thereto.

The present invention may be better understood by reference to the following non-limiting Example, which is provided as exemplary of the invention. The following Example is

presented in order to more fully illustrate the preferred embodiments of the invention. It should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE

The mu opioid receptor is the major target for clinically important opioid alkaloids including morphine, methadone, fentanyl, and other opioid drugs (1,3), as well as for endogenous opioid peptides such β-endorphin, Met-enkephalin-Arg-Phe, and the recently identified endomorphins (5). Furthermore, it is the major molecular site of action for heroin (2,6). Rapid activation of the mu opioid receptor, such as occurs in the setting of drug abuse, results in a euphoric effect, thus conferring the reinforcing or rewarding effects of the drug, contributing to the development of addiction. Clinical observations have suggested that individuals have varied sensitivity to opioids, suggesting potential variability in the receptor protein and gene.

Molecular cloning of the mu opioid receptor (7-9) has made it possible to determine potential sequence polymorphism, as shown by a recent study (10). To further identify polymorphisms of the mu opioid receptor, a PCR-based strategy was used to amplify the coding regions of the mu opioid receptor gene, and to determine the DNA sequence of the amplified exons. Using this method DNA samples were sequenced from 450 subjects including both former heroin addicts in methadone maintenance treatment and individuals with no history of opiate or non-opiate drug dependence, as well as individuals with non-opiate drug abuse and dependence.

By sequencing PCR-amplified DNA from the study subjects, it was determined that the previously reported sequence for the human mu opioid receptor (8,9) was the most common allele found in the study population. Five new polymorphisms were also identified: T67C, T124A, C153T, G174A, or 187INS:GGC, of which C153T and G174A are silent, T67C results in Ser23Pro, T124A results in Ser42Thr, and 187INS:GGC results in the insertion of a Gly residue after Gly 63. For the purpose of this study, the term "most common" was used to denote the predominant mu opioid receptor allele and the corresponding receptor that was

originally reported by cDNA cloning (8,9), and the term "variant" to denote the allelic genes/receptors containing polymorphic variations.

The results of sequencing of the PCR-amplified mu opioid receptor genes are shown in the following electropherograms. **Figure 7A - 7B** show an electropherogram of the mu opioid receptor DNA from an individual heterozygous for both the A118G and the T124A single-nucleotide polymorphisms. Figure 7A is the sequence of the (+) strand; figure 7B the (-) strand.

Figure 8A - 8B show an electropherogram of the mu opioid receptor DNA from an individual heterozygous for the C153T single-nucleotide polymorphism. Figure 8A is the sequence of the (+) strand; figure 9B the (-) strand. **Figure 9A - 9B** show an electropherogram of the mu opioid receptor DNA from an individual heterozygous for the G174A single-nucleotide polymorphism. Figure 9A is the sequence of the (+) strand; figure 9B the (-) strand. **Figure 10A - 10B** show an electropherogram of the mu opioid receptor DNA from an individual heterozygous for the 187INS:GGC polymorphism, in which a GGC codon is inserted after position 187. Figure 10A is the sequence of the (+) strand; figure 10B the (-) strand. **Figure 11A - 11B** show an electropherogram of the mu opioid receptor DNA from an individual heterozygous for the T67C single-nucleotide polymorphism. Figure 11A is the sequence of the (+) strand; figure 11B the (-) strand.

Based on these results, the sequence alterations in the four polymorphisms of the invention were obtained. **Figure 1A-1B** show the nucleic acid (1A) and protein (1B) sequence of the most common allele (i.e., wild type) of the mu opioid receptor (SEQ ID NO:1 and SEQ ID NO:2, respectively) (GENBANK accession number L25119). **Figure 2A-2B** show the DNA (2A, SEQ ID NO:3) and protein (2B, SEQ ID NO:4) sequence of the most common allele of the mu opioid receptor with the T67C polymorphism. As noted above, any of the other present or previously described mu opioid receptor polymorphisms may also be present; these and the following sequences merely show the wild-type DNA and protein sequences with the one polymorphism exemplified. **Figure 3A** shows the DNA sequence (SEQ ID NO:5) of the most common allele of the mu opioid receptor with the T124A polymorphism. Likewise, **Figure 4** shows the DNA sequence (SEQ ID NO:6) of the most common allele of the mu opioid receptor

with the C153T polymorphism, **Figure 5A-5B** show the DNA (5A, SEQ ID NO:7) and protein (5B, SEQ ID NO:8) sequence of the most common allele of the mu opioid receptor with the C174A polymorphism, and **Figure 6A-6B** show the DNA (6A, SEQ ID NO:7) and protein (6B, SEQ ID NO:8) sequence of the most common allele of the mu opioid receptor with the 187INS:GGC polymorphism.

By sequencing PCR-amplified DNA from the study subjects, it was determined that the previously reported sequence for the human mu opioid receptor (8,9) was the most common allele found in the study population. Five different polymorphisms were also identified. For the purpose of this study, the term "most common" or "prototypic" was used to denote the predominant mu opioid receptor allele and the corresponding receptor that was originally reported by cDNA cloning (8,9), and the term "variant" to denote the allelic genes/receptors containing polymorphic variations.

Study subjects and procedures. Addictive disease patients, specifically long-term heroin addicts currently in chronic methadone maintenance treatment, and normal control subjects with no history of any drug or alcohol abuse, and individuals with non-opiate drug abuse and dependence were extensively characterized with respect to drug abuse, the addictive diseases, psychological and psychiatric profiles, and medical and ethnic family backgrounds. Unrelated study subjects who were former heroin addicts were referred from methadone treatment clinics in the greater New York City area, primarily those associated with The Biology of Addictive Diseases Laboratory located at The Rockefeller University. These clinics are the Adolescent Development Program and Adult Clinic at the New York Hospital-Cornell Medical Center. Previously heroin-addicted patients admitted to the study conformed to the federally regulated criteria for admission to a methadone maintenance program, that is, one or more years of daily multiple-dose self-administration of heroin or other opiates with the development of tolerance, dependence, and drug-seeking behavior (38). Current or prior abuse of other drugs was not used as an exclusion criterion for this group as long as opioid abuse continued to be the primary diagnosis.

Unrelated healthy volunteer subjects were recruited primarily through posting of notices and

newspaper advertisements or referral by physicians or staff at the Rockefeller University Hospital. Individuals with continuing drug or alcohol abuse or prior extended periods of regular abuse were also studied.

Both addictive disease patients and normal volunteers admitted to the study were assessed by a psychiatrist or research nurse with several psychiatric and psychological instruments as well as the Addiction Severity Index (39). Study subjects were also administered a detailed personal and medical and special addictive disease questionnaire as well as a family history medical and addictive disease questionnaire designed to provide information regarding substance abuse and major mental illness of first and second degree relatives. Study subjects provided detailed information regarding family origin and ethnic background, including country or geographic area of birth. This information was obtained for both the study subjects themselves and their immediate ancestors (parents, grandparents and great-grandparents), to the extent that the information was known by the study subjects. Study subjects were classified into five groups: African-American, Caucasian, Hispanic (Caribbean and Central or South American origin), Native North American, and Other. The detailed ancestral information collected by the family origin questionnaire allowed classification of study subjects into defined categories. Following psychiatric and behavioral assessment and informed consent and family history acquisition, venipuncture on the study subject was performed, and a blood specimen was taken. Blood samples were processed for DNA extraction and EBV transformation to create stable cell lines that were stored for future studies. All blood samples were coded; the psychiatrists and nurses who performed psychiatric and psychological assessments were blind to the genotypes of the study subjects, and the identity and categorization of the study subjects was unknown to the laboratory research personnel.

Exon amplification and sequencing. Sequences for the non-coding regions of the human mu opioid receptor gene were used to design PCR primers for the sequencing of the first exon. Exon 1 forward primer sequences were based on the 5'-untranslated region of the receptor (9). Only one reverse primer was used for exon 1. The PCR reactions were performed with 50-100 ng of genomic DNA. DNA polymorphisms were confirmed by both manual and automated sequencing on both strands, forward and reverse.

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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